# APPENDIX 7

# Letters to the Editor

## MASCULINIZATION OF THE FEMALE INFANT ASSOCIATED WITH ESTROGENIC THERAPY ALONE DURING GESTATION: FOUR CASES*

To the Editor:

Masculinization of the female infant has in some instances been attributed to the antenatal exposure of the fetus to androgens from several sources. Virilizing hormones may arise: 1) from the fetal adrenal in the adrenogenital syndrome due to congenital adrenocortical hyperplasia; 2) from virilizing tumors in the mother (arrhenoblastoma); 3) from the administration of primarily androgenic hormones to the mother during pregnancy; and 4) from certain "progestins" administered to the mother during pregnancy. This report concerns 4 female infants and children with some degree of masculinization who were recently seen in 3 different clinics. In each instance it was determined that the mother had received diethylstilbestrol *alone* during pregnancy. There were no other factors discovered to account for these changes and it was considered that the estrogenic hormone probably played some role in producing the effects described.

### CASE REPORTS

*Case 1*

*J. L.* was seen at The Children's Hospital of Philadelphia, when 4¾ years of age, because of abnormal genitalia present since birth. The mother had had 4 previous pregnancies, 3 of which had terminated spontaneously between three and six months' gestation. The fourth was uneventful and productive of a "perfectly normal" female, now aged 7½ years. The patient under consideration was the product of a fifth pregnancy during which diethylstilbestrol had been administered to the mother from the seventh to the twentieth week, in a dosage of 5.0 to 50.0 mg. daily. Progesterone or "progestins" had not been used. No other unusual hormones or medications had been administered to the mother. At the time of the first examination, the patient was in the fiftieth percentile for both height and weight. She displayed no evidences of masculinization aside from the changes in the genitalia. The clitoris was obviously enlarged, measuring 2.5×1.0 cm., with a redundant foreskin and a dimple on the glans which failed to admit a fine

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catheter. The labioscrotal folds were fused to the base of the phallus (Fig. 1), despite an operation which the mother stated had been performed at an earlier age in order to open the vagina. The patient's mother had at no time experienced virilization, but she was not further studied because of severe neurosis and undue concern for her child. A vaginogram performed on the patient via the small orifice, revealed a vagina, cervix uteri, and urethra; the latter was slightly displaced posteriorly. The nuclear chromatin pattern was female. No further studies were permitted.

FIG. 1. *Case 1*

FIG. 2. *Case 2*.

*Case 2*

*B.R.*, a 7-year-old girl, was seen at The Children's Hospital of Philadelphia because of an enlarged clitoris present since birth. The parents had always been aware of this and had repeatedly procrastinated because of what they believed to be unbearable prospects. The mother's first 2 pregnancies terminated at the third and fourth months, respectively. Her third pregnancy was successful, presumably without treatment, and productive of a normal male, now aged 13 years. The patient was born after a pregnancy characterized by bleeding during the fourteenth week, for which reason the mother received diethylstilbestrol, 25 mg. daily, through the twentieth week. No other hormones, including progesterone or its analogues, had been employed. On physical examination, the child was normal in size for her age (about the sixtieth percentile for height and weight) and as yet sexually immature. There was no hirsutism. The significant physical findings were confined to the genitalia (Fig. 2). The clitoris was obviously much enlarged, measuring 4.5×1.9 cm., with a firm corpus and redundant foreskin. There was only minimal fusion posteriorly to the labioscrotal folds, the vaginal orifice appeared

adequate, and the urethral orifice was easily seen in its normal position. Nonetheless a vaginogram was performed, which revealed a normal sized vagina and cervix uteri. The uterus, normal in size, was palpable on rectal examination. The bone age was normal. The nuclear chromatin pattern was female. Excretion of 17-ketosteroids, determined on several specimens of urine, ranged from 0.6 to 3.5 mg. per twenty-four hours. The mother was in good health and did not exhibit virilization during pregnancy. Her urine contained 10.7 mg. of 17-ketosteroids and 0.8 mg. of pregnanetriol in a 24-hour specimen at the time the patient was first observed by us.

FIG. 3 *Case 3.*

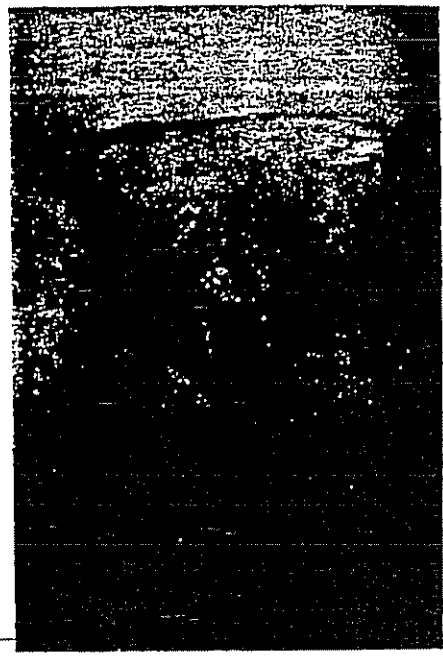

FIG. 4 *Case 4.*

*Case 5*

*C.J.* was seen at the Babies Hospital in New York City when 2 months of age, because of abnormal external genitalia. Owing to a miscarriage at the third month during the only previous pregnancy, the mother received diethylstilbestrol, 10 mg. daily, during the thirteenth week of this pregnancy; the dosage was gradually increased to 75.0 mg. daily during the twenty-fifth week. No other hormones were administered. The patient was born at the end of the thirtieth week of gestation. The birth weight was 1150 Gm. When examined at 2 months of age she weighed 2380 Gm. At this time the pertinent physical findings were limited to the external genitalia. The clitoris was enlarged and measured 3.2×1.0 cm.; it was covered by a prominent preputial fold (Fig. 3). The labia majora were fleshy and conspicuous but there was no fusion. Separate urethral and vaginal orifices were identified. Oral mucosal smears showed female-type nuclei and the 24-hour excretion of 17-ketosteroids was 0.1 mg. A 24-hour specimen of the mother's urine was examined and contained 4.2 mg. of 17-ketosteroids and 6.0 mg. of corticoids.

*Case 4*

*E. V.*, at 7 days of age, was admitted to St. Christopher's Hospital for Children in Philadelphia because of abnormal genitalia. The mother was treated with 5 mg. of diethylstilbestrol twice daily between the fourth and ninth week of pregnancy because of severe abdominal cramps. No other hormones were administered during pregnancy and the mother had not had any signs of virilization. The cramps subsided a few days after institution of therapy. The patient had a brother and sister who were normal and there was no family history of congenital abnormalities. Physical examination revealed clitoral enlargement (1.5 cm.) and labioscrotal fusion to the base of the phallus (Fig. 4). The 24-hour 17-ketosteroid excretion was 0.6 mg. The chromosomal sex was female. Injection of radio-opaque media into the urogenital sinus resulted in filling of the bladder, but a vagina was not demonstrated. Exploratory operation revealed a normal uterus, tubes and ovaries—the latter proved by biopsy. There has been no progressive virilization of the infant and growth and development have been normal.

## DISCUSSION

Congenital adrenocortical hyperplasia, arrhenoblastoma in the pregnant woman, and the administration of primarily androgenic hormones as factors in the masculinization of the female infant have been considered in a monograph by Grumbach and Barr (1). More recently, Wilkins *et al.* (2), Jones (3), and Hayles and Nolan (4) have called attention to similar effects produced by the administration of certain progestins. Wilkins *et al.* (2) pointed out the similarity in structure of some progestins and androgenic hormones; however they attributed their findings to some unusual metabolic disposition of these compounds which may produce androgenic substances. It should be noted that 10 of 21 cases reported by Wilkins and associates (2) received estrogens (diethylstilbestrol in 9) as well as progestins.

The incrimination of predominantly estrogenic hormones in the 4 cases reported here seems paradoxical. However there is some basis in experimental embryology for this occurrence. Although it is difficult to establish these changes as due to diethylstilbestrol, all other known factors have been ruled out. The mothers received no other hormones. The true adrenogenital syndrome was adequately ruled out in each of the children on both laboratory and clinical grounds; in none of the patients was masculinization progressive. None of the mothers presented clinical evidence of virilization and hence it appears unlikely that endogenous maternal hormones, in abnormally large quantity, played any role. In 2 of the mothers who were studied, the daily excretion of 17-ketosteroids was normal.

Much attention has been given in experimental embryology to the alteration of sexual development by the administration of estrogenic or androgenic hormones during gestation. Ordinarily, attention has been focused on the ability of one group of hormones to bring about homologous effects on fetuses of the opposite sex. In 1939 Greene *et al.* (5) reported

paradoxical effects of estrogens on the sexual development of the female rat. As expected, when estradiol was given to pregnant rats, the male offspring were feminized, with inhibition of development of the epididymis, vas deferens, seminal vesicle and prostate; the vagina, parts of the uterus, and nipples persisted. Surprisingly, the female offspring showed paradoxical effects; there was inhibition of development of the lower vagina, with a small opening at the caudal base of the cleft phallus; in addition, there was inhibition of the ovarian capsule, partial preservation of wolffian-duct structures, and even seminal vesicle formation in a few. Later, Greene and his colleagues (6) employed diethylstilbestrol as well as estradiol, presumably with similar results. Moore (7) reported the stimulation of wolffian ducts by estrogens in the oppossum, and this occurred in both sexes—an observation differing somewhat from that in the rat. However, enlargement of the clitoris was not described in these experiments. Any explanation for these curious results was lacking. A direct teratogenic effect of the large doses of estrogens on the genital primordia must also be entertained.

The work of Lacassagne in 1927 (8), demonstrating an increase in adrenal size following the administration of estrogens to rats, has been repeated by many others with similar results. This observation has also been recorded for the guinea pig, mouse, cockerel and other species. In 1945 Vogt (9) demonstrated that the administration of hexestrol (a synthetic estrogen, related to diethylstilbestrol) caused a complete loss of lipids in the adrenal gland of the rat within five days, with an increase in the size of the organ and general intolerance to stress akin to adrenocortical insufficiency. Subsequently Vogt (10) described a diminution in the secretory rate of corticosterone by the rat's adrenal following the administration of hexestrol; progesterone alone did not produce this result. The rabbit was not so affected by hexestrol. Clayton and Hammant (11) recently described changes in the adrenal of the rat (but not the guinea pig) following administration of stilbestrol, with an increase in the size of the gland. They suggest interference with the normal secretion of steroids. Witschi (12) described adrenocortical hyperplasia in the larvae of 3 species of Rana when estrone, estradiol, ethinyl-estradiol or equilinin were added to the water in the aquaria. Smaller doses of the estrogens produced feminization of males, whereas larger doses also led to masculinization of genetic females. He suggested that estrogens might lead to cortical gonadal deterioration, but in view of the extreme adrenal hyperplasia, he suggested adrenal androgens as a factor and hence designated his observations as the "experimental adrenogenital syndrome." The presence of the pituitary is essential to the production of adrenal hyperplasia, but not necessarily to the production of genital effects. Although Witschi was unable to induce similar sexual alterations with stilbestrol, it is noteworthy that in Rana the adreno-

cortical hyperplasia does not occur with this compound. Little is known of the effects of large doses of stilbestrol on the human maternal or fetal adrenal cortex during pregnancy. Is it possible that temporary fetal or maternal adrenocortical hyperplasia with the production of androgens occurs under the influence of large doses of stilbestrol during pregnancy in selected instances?

Cases 1 and 4 were the most severe, and in these stilbestrol was administered earlier in the course of pregnancy. This suggests some relationship with the embryonic sequence of development of the genitalia as invoked for the effects of progesterone by Wilkins *et al.* (2). The effects described in our cases must be regarded as unusual. At the Joslin Clinic, 950 diabetic pregnant women have been treated with female sex hormones without any apparent influence on the genitalia of the offspring (L. Gillespie, personal communication); 700 were given stilbestrol, 25 mg. per day initially and eventually 125 mg. daily. Treatment was generally begun very early in gestation and continued until delivery. It must be inferred (if stilbestrol played a role in the patients described here), that only a rare fetus or pregnant woman is susceptible to estrogens in a manner leading to virilization. The nature of the susceptibility is unclear. Analogous contrary evidence exists for the culpability of certain progestins in effecting masculinization of the female infant as described by Wilkins *et al.* (2). Rakoff (personal communication) has employed these same hormones through the years without observing the effects described by others.

Although feminization of the male fetus by estrogens has been described in some species (5–7), it has not previously been reported in man. Recently, 1 such possible event in man has been called to our attention (13).

It should be added that so-called "non-adrenal" or "non-hormonal pseudohermaphrodism" has occasionally been described. We cannot incriminate estrogens in our 4 cases with finality at this time. On the other hand, cases previously assigned to the non-hormonal group have subsequently been associated with the use of progestins in one of our clinics (A.M.B.).

## SUMMARY

Four instances of masculinization of the female infant, attendant upon the administration of stilbestrol to the mother during pregnancy, are described. No other causative factors could be recognized. The mothers received no other hormones, none was suffering from a virilizing syndrome, and the infants were excreting normal amounts of 17-ketosteroids. Such an occurrence in man is regarded as unusual in the face of the widespread use of estrogenic hormones during gestation, but it is *not* without its precedent in experimental embryology, in which field the paradoxical effects of estrogens on the female infant have been described. A peculiar and rare suscepti-

bility of the human adrenal to the action of stilbestrol is suggested. Experimental evidence points to adrenal hyperplasia consequent to the administration of estrogens as a possible factor, although a non-hormonal teratogenic action is not ruled out. It is expected that other similar cases of masculinization associated with the use of estrogens during pregnancy will be discovered.

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## *Addendum*

Dr. Roy Hertz of the National Institutes of Health has observed clitoral enlargement in a female child who had ingested estrogen-contaminated vitamins; and rapidly subsiding clitoral hypertrophy in a newborn female whose mother had received stilbestrol during the pregnancy. Dr. Judson J. Van Wyk, University of North Carolina, reports to us that he has observed moderate clitoral hypertrophy and posterior fusion of the labioscrotal fold in an infant born of a mother who received micronized stilbestrol (25 mg. daily) beginning about the eleventh or twelfth week of gestation; he states that the treatment "was well toward the end of the period during which one would anticipate anatomical effects on the urogenital sinus and would expect the defects to be mild."

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## IDENTIFICATION OF "FREE" TETRAHYDROCORTISONE IN HUMAN PERIPHERAL PLASMA*

TO THE EDITOR:

In two previous reports (1, 2) we have suggested that even in certain "normal" persons much of the Porter-Silber reactive material detected in plasma may *not* be $\Delta^4$-3-ketocorticosteroids, but may possibly be "free" tetrahydro E or tetrahydro F, and have cautioned against the uncritical acceptance that the plasma level of "free" Porter-Silber reactive material reflects the true state of adrenocortical activity. We are now presenting evidence that tetrahydro E in the unconjugated state is indeed present in "normal" human peripheral plasma.

To attain levels of corticosteroids in human plasma sufficiently high to permit isolation of individual compounds for definitive identification, 10 liters of fresh human bank plasma were processed. Upon preliminary assay of this plasma, the concentration of "free" Porter-Silber reactive material exceeded that of the "free" $\Delta^4$-3-ketocorticosteroid content as measured by the isonicotinic acid hydrazide (INH) reaction by 5 micrograms ($\mu$g.) per 100 ml. This would result in a maximal potential expectancy of 500 micrograms of tetrahydro E or tetrahydro F in 10 liters of plasma.

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